



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: <b>PCT/GB93/02290</b> (22) International Filing Date: <b>8 November 1993 (08.11.93)</b> (30) Priority Data: <b>9225307.9</b> <b>3 December 1992 (03.12.92)</b> <b>GB</b> (71) Applicant (for all designated States except US): <b>TECHNE (CAMBRIDGE) LIMITED [GB/GB]; Duxford, Cambridge CB2 4PZ (GB).</b> (72) Inventors; and (75) Inventors/Applicants (for US only): <b>NORTON, David, Robert [GB/GB]; 6 Rotterdam Drive, Avon Village, Christchurch BH23 1HB (GB). REID, Mark, Anthony [GB/GB]; 7 Waterside, Mudeford, Christchurch BH23 3NE (GB).</b> (74) Agent: <b>JOHNSON, Terence, Leslie; Edward Evans &amp; Co., Chancery House, 53-64 Chancery Lane, London WC2A 1SD (GB).</b>		(81) Designated States: <b>AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b>  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: <b>CLOSURE MEANS, CONTAINERS AND METHODS OF CLOSURE</b>		
(57) Abstract		
<p>           A closure means for containment devices such as reagent containment devices (RCDs) comprising a multilayer film (10) having at least two layers, an uppermost first layer (11) and adjacent to the first layer a second sealing layer (12), said first layer comprising a heat resistant barrier being substantially impermeable to gas and moisture. The film can be in the form of a strip or sheet and can be applied to for example microtubes, and multiwell plates or strips in order to hermetically seal them and prevent reagent loss through evaporation during treatment or spillage during handling or storage. There is further provided a method for sealing RCDs comprising applying a film as described using heat and/or pressure, forms of RCDs useful for said method, and kits comprising closure means and RCDs.         </p>		

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CLOSURE MEANS, CONTAINERS AND  
METHODS OF CLOSURE

The invention relates generally to closures for containers, and to containers themselves, and to methods for sealing containers.

More particularly, the present invention relates to closure means for containers suitable for use in all situations where a heat resistant, gas and moisture resistant seal is required, such as for example in food storage and preparation, and preservation of objects, for example archaeological artifacts.

One particular area of applicability is in the fields of molecular biology and genetics and to the various techniques of DNA amplification, whereby different reagent liquids are mixed together, and utilized in combined small volumes typically in the range 5.0 $\mu$ L to 500 $\mu$ L. These enzymatic mixes are then subjected to thermal cycling between temperatures of around -4°C to 98°C.

A typical thermal cycling protocol consists of sample preparation at 4°C followed by thirty amplification cycles with each cycle consisting of 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C.

Although there are numerous protocols in operation, with many variations of time and temperature, all cycles contain a high temperature denaturation phase, followed by a low temperature annealing phase and intermediate extension phase, as referred to above.

In reactions termed sequencing however, the low and intermediate phases are often combined with the effect that each cycle consists of two rather than three separate temperatures.

The general handling procedure and practice for all thermal cycling is to dispense a reagent mixture into a suitable reagent containment device (RCD) and subsequently place the RCD onto or into a programmable thermal cycler (PTC).

A number of alternative programmable thermal cyclers (PTCs) are commercially available. All permit the selection and cycling of all times and temperatures up to 99.9°C.

Heating and cooling is produced by a combination of accurately controllable means. Most have machined or cast aluminium heat blocks, some have copper blocks whilst some have blocks manufactured from electro-deposition of silver. Some, even, consist of programmable water bath arrangements.

Heating in such PTCs is produced by devices such as cartridge heaters, peltier thermoelectric elements, halogen bulbs and blown hot air. Cooling in such PTC's is produced by blown cool air, thermo-electric elements or pumped cooling water.

The reagent mixtures placed within the RCDs are cooled and heated by direct conduction from the different thermal block materials and each PTC is manufactured so that one or more RCD fits closely into its particular thermal block format.

Temperature accuracy and speed of cycling depends on a number of factors including, thermal conduction and capacity of thermal block, section thickness of RCD, volume of sample and intrinsic heat/cool rate of PTC.

Presently all thermal cycling reactions utilize disposable synthetic RCDs that are termed microcentrifuge tubes or microtubes (MTs), thin walled tubes and strips

(TWTs) or multiwell plates and strips (MPs). All these RCDs are either injection moulded or thermoformed from familiar synthetic thermoplastics. The essential requirement of the RCD material when used for thermal cycling at elevated temperatures is that the material resists high temperature cycles such as those employing temperatures in the range 95-98°C. For this reason most RCDs used are currently manufactured in polypropylene or polycarbonate.

MTs and TWTs are typically conical or tapered at their base with parallel sides when viewed in section and have sample volumes of between 0.2 and 2.0 ml. MP's possess a plurality of spaced sample wells, universally produced as an integral array of 96 wells disposed 12 X 8, typically, 25µL in volume, and are usually, 'flat', 'U' or 'V' shaped in profile. Multiwell strips are produced with one or two rows of 8 or 12 of these similarly profiled wells.

It is generally a requirement that RCDs are provided with a means to prevent sample loss through evaporation or spillage whilst undergoing processes such as thermal cycling. Any moisture or gas loss at elevated temperature can lead to, at best, erroneous results, through chemical and solution concentration, and at worst, total reaction failure.

A further consequence of sample loss is the need to use more reagents than would otherwise be necessary for consistent and accurate results. This is expensive, due to the high cost of the polymerase reagents and other enzymes that are utilized in such reactions.

One method of preventing sample loss currently in use in MTs is the provision of an integral press fit hinged lid. This design however fails to prevent evaporation.

In TWTs sample loss is prevented by a separate cap-strip design that seals by heavy external pressure and deformation. The force is supplied, in this instance, by a propriety design of PTC which has a heavy screw-down lid incorporated into its design. However, this makes processing using this machine both costly and time consuming.

In MPs no sealing system is available that satisfactorily prevents sample evaporation and loss at the elevated temperature experienced during thermal cycling protocols.

All existing RCDs, as described above, except the TWTs, utilize the external addition of a mineral oil overlay to prevent sample loss through evaporation.

A problem when using mineral oil overlays is that the additional volume of oil slows the reaction of the reagents giving rise to inconsistent and corrupted results. Contamination of the sample is also possible. This can occur during thermal cycling and during sample extraction as the sample is accessed through the mineral oil overlay. Lastly, the preparation of the overlay and extraction of the sample through the overlay is time consuming, and, expensive.

It is accordingly an object of the present invention to seek to mitigate such problems.

Thus according to a first aspect of the present invention there is provided a closure means for a containment device, the closure means comprising a polymeric film, said film being characterised by a heat resisting barrier layer and a sealing layer, the barrier layer being substantially impervious to gas and moisture penetration.

In one preferred embodiment the containment device comprises a reagent containment device (RCD).

It is preferred that the layers remain substantially adjacent one another in use, and that they are adhered together. Furthermore, the barrier layer is preferably formed from one or more high temperature formable polymer, such as polyesters, polyamides, polyimides, and polymethylpentenes. The sealing layer is preferably formed from one or more of polypropylenes, polycarbonates, polystyrenes, polymethylmethacrylates, polyvinylchlorides, and amorphous polyesters.

It is also preferred that the closure means, comprises at least one additional layer having heat- and moisture- insulating properties, such as for example an additional layer comprising aluminium.

The closure means according to the invention, produced by lamination or co-extrusion in the form of a tape or sheet.

According to a second aspect of the invention, there is provided a method for sealing a container device such as an RCD comprising applying a closure means, according to the invention, said application being by applying heat to said closure means, or heat and pressure.

According to a third aspect of the invention, there is provided an RCD comprising containment means, and closure means according to the invention, characterised in that said containment means comprises a polymeric material and said closure means is non-integral and comprises a polymeric material including at least the constituents of the polymeric material of said containment means.

The RCD may comprise a plurality of containment means, and said containment means may be substantially U-shaped in vertical section, or substantially V-shaped in vertical section.

According to a fourth aspect of the invention, there is provided a multiwell RCD, comprising a plurality of containment means and a carrier comprising a plate or tray, said containment means forming wells or depressions in said plate or tray, and further comprising an annular ring surrounding each well or depression.

According to a fifth aspect of the invention, there is provided a microtube RCD, comprising a tube having a sealing surface comprising a flange, said tube comprising substantially parallel side walls, and a base section, said flange providing support means for said sealing surface.

It is preferred that the RCDs according to the invention, have a capacity of from 5 micro litres to 100 ml, with a capacity of about 250 micro litres, and a wall thickness from 0.4 to 20mm.

According to a sixth aspect of the invention there is provided a kit comprising a containment device and closure means, according to the invention.

Such a kit may further comprise a dispensing means for said closure means.

There is further provided a kit comprising a containment device, wherein the containment device is an RCD according to the invention.

Closure means and containers suitable for use with said closure means embodying the invention are hereinafter described, with reference to the



accompanying drawings in which:

Fig. 1A is a cross sectional and perspective view of a portion of a film according to the present invention;

Fig. 1B is a cross sectional and perspective view of a portion of an alternative embodiment of the present invention;

Fig. 2 is a perspective view of an RCD according to the present invention;

Fig. 3 is a cross section taken along line X-X' in Fig. 2;

Fig. 4 is a cross sectional view of a closure means according to the invention illustrating the appearance of a microtube RCD after having been sealed by the application of heat or heat and pressure to a closure means according to the invention;

Fig. 5 is a plan view of a multiwell plate RCD according to the present invention.

Fig. 6 is a cross sectional view along line Y-Y' in Fig. 5;

Fig. 7 is a cross sectional view of a multiwell plate RCD having flat microwells and solid wall sections and annular sealing rings of similar inside diameter as the microwells; and

Fig. 8 is a cross sectional view of a multiwell plate RCD and closure means according to the invention after they have been sealed by the application of heat or heat and pressure.

Reference is now made to the drawings wherein like numerals are used to designate like parts throughout. Specific reference is made to Figs. 1, 2 and 3 which comprise cross sectional views of the sealing film 10, a perspective of a lid-less microtube RCD 20 and a cross section of the same microtube RCD, respectively.

The sealing film 10, that can be produced in either cut-sheet form or in reel form, is illustrated as generally comprising a multilayer polymeric sheet or reel consisting in part of an uppermost heat resisting, barrier layer 11 in adjacent association with a lowermost (as viewed) sealing layer 12. The sealing film 10 can be produced by techniques such as lamination or co-extrusion. In either case the layers 11 and 12 are generally separated by a coated adhesive layer 13 that serves to hold upper and lower layers 22 and 12 together.

The barrier layer 11 can be formed from a variety of polymers, such as polyesters, polyamides, polyimides, polymethylpentenes and other high temperature formable polymers. The sealing layer 12 may be formed from polymers that are chemically similar to the polymers that are used in the injection moulding and thermoforming of the aforementioned RCDs 20. These are generally, polypropylenes, polycarbonates, polystyrenes, polymethylmethacrylates, polyvinylchlorides and amorphous polyesters.

The chemical nature of the barrier layer 11 bestows enhanced physical properties such as strength, rigidity and decreased gas and moisture permeability to the multilayer structure. When there is a similarity between the chemical nature of the sealing layer 12 and RCD 20 a wide range of pressure and heat resisting sealabilities is produced.

Sealing layer 12 hermetically seals to the flat top surface 21 of the microtube

RCD. The manner of sealing can be either permanent or peelable, such that the contained reagents are accessed either by puncturing the sealing film 10 or by complete removal of the sealing film 10 by simple peeling means.

The microtube RCD 20 is illustrated as comprising in the embodiments a small injection moulded or thermoformed lid-less tube or well having a flat top sealing surface 21, incorporating a holding flange or lip 22, with parallel sides 23 terminating in a conical base section 24. More generally the microtube RCD 20 can be constantly tapered and have either a 'V', 'U' or 'flat' base section 24.

Typical dimensions for such microtube RCDs would be an internal volume of around 250 $\mu$ L, wall thickness 23 and base thickness 24 of about 0.4mm and height of about 20mm. Many variations are possible up to capacities of about 100ml, and heights of about 150mm.

Microtube RCDs 20 can be produced and sealed either individually or as strips. In both instances they can be assembled into a variety of multiwell formats including the familiar and universally adopted 96 well (12 X 8) configuration.

It is advantageous that the RCD top surface 21 is small in comparison to the overall area of the sealable film 10 to which it is sealed. In this way sealing film 10 and microtube RCD 20 are fused, permanently or peelably, quickly and consistently under the application of heat and pressure with no detrimental effects to contained polymers or other liquid reagents.

The sealing film 10 can also incorporate additional layers such as aluminium foil layers 14 or aluminium layers 14 that have been vacuum deposited. In either case these layers may be situated between layers 11 and 13 and bestow decreased gas and moisture transmission characteristics to the sealing film 10.

Barrier layers 11 are generally 5 to 100 $\mu$ m in thickness, aluminium layers are generally 1 to 200 $\mu$ m in thickness and sealing layers 12 are generally 10 to 500 $\mu$ m in thickness. The overall dimensions of the sealing film permits sealing times of around 0.5 to 3.0 seconds under the application of heat or heat and pressure when utilized in conjunction with microtube RCDs 20 according to the present invention.

Reference is now made to Fig. 4 in which a reagent mixture 30 is contained within the microtube RCDs. The sealing film 10 is applied across a number of microtubes 20 before sealing the tubes 20 and film together under the application of heat and pressure. Sealable layer 12 and microtube RCD top surface 21 are melted together using heat and pressure, being similar chemically and therefore compatible, to produce a pressure resistant and hermetic seal suitable for either thermal cycling, handling or prolonged storage. The reagent mixture can be accessed by either puncturing the entire multilayer sealing film 10, or peeling the entire film away from the microtube RCDs 20.

Reference is now made to Figs. 5, 6 and 7 which illustrate a further embodiment of the present invention where the sealing system is applied to an improved design of multiwell plate RCD. Fig. 5 shows a plan view of a typical multiwell plate RCD 40, generally containing either 96 regularly spaced microwells 41, or multiples thereof such as 192, 384 or 864. Multiwell strip RCDs can also be produced in single or double strips containing multiples of 8 or 12 microwells. There are provided raised annular sealing rings 42 concentric with each microwell 41 and having flat top surfaces 42 with parallel or tapered sides 43. The annular sealing rings 42 are typically 0.25 to 1.00mm in height and with a radial thickness of 0.5 to 2.00mm and can possess diameters either similar to the microwells or be slightly larger in diameter.

The microwells 41 can comprise either largely parallel sides 43, with thin cross section wall thickness, formed more usually by the technique of thermoforming, terminating in a conical base section 44 or be formed from a largely solid cross section 46, formed more usually through the technique of injection moulding. More generally the multiwell plate RCDs 40 can have microwells 41 that are constantly tapered and have either 'V', 'U' or 'flat' base sections 44.

The sealing film 10 is sealed to the multiwell plate RCD 40 by the simple application of heat, or heat and pressure which fuses both together either permanently or peelably, by melting and welding of the sealing layer 12 contained within the film and the annular sealing ring 42.

The melting of the annular ring 42 is enhanced by its low surface area which makes for easy and fast sealing. The melted area creates a small annular weld zone 42a which acts as a barrier to liquids and is sufficiently strong to resist the heat and pressures produced during thermal cycling.

The annular sealing ring 42 also serves as a barrier to contamination and spillage when multiwell RCDs are being filled with reagent mixtures.

A microtube RCD 20 or microwell 41 having an internal volume of around  $200\mu\text{L}$ , height 20mm and wall thickness 0.5mm, performed repeatable and consistent amplification experiments, including 'sequencing' type reactions on reagent mixtures in the range from  $1.00\mu\text{L}$  to  $200.00\mu\text{L}$ .

The invention will now be further illustrated with reference to specific examples.

Examples:

(1). A sealing film consisting of a multilayer assembly of 12 $\mu$ m polyester and 50 $\mu$ m polypropylene laminated with an adhesive layer were heat and pressure sealed to an array of 96 polypropylene microtube RCDs containing between 1.0 $\mu$ L and 200 $\mu$ L of polymerase reagent mixtures. No oil overlays were used. The tubes were given 30 amplification cycles, each cycle consisting of 1 min. at 95°C, 1 min. at 72°C and 1 min. at 55°C in a commercial PTC. After the experiment the mixture was measured for sample loss which was negligible. Subsequent analysis confirmed the reaction proceeded successfully.

(2). A sealing film, similar to the above example was heat and pressure sealed to a multiwell plate RCD consisting of an array of 384 flat bottomed wells of 70 $\mu$ L volume injection moulded from polypropylene. Each well contained between 1.0 $\mu$ L and 50.0 $\mu$ L of polymerase reagent mixture. The multiwell plates were thermally cycled by being robotically placed into two water baths held at 72°C and 95°C, respectively. 30 cycles were completed each cycle consisting of 1 min. at each temperature in each bath. No oil overlays were used. No sample loss was recorded. Subsequent analysis confirmed that the amplification reactions had proceeded successfully.

(3). A thermoformed polycarbonate multiwell RCD consisting of 96 individual wells of approx. 200 $\mu$ L capacity was fused to a sealing film consisting of 20 $\mu$ m polyester/60 $\mu$ m polycarbonate in association with an adhesive layer. Reagent samples of 50 $\mu$ L volume were thermally cycled in the manner of example (1). Similar and successful results were produced.

(4). A film consisting of 10 $\mu$ m polyester, 20 $\mu$ m aluminium foil and 50  $\mu$ m polyethylene was fused by the application of pressure and heat to a similar array of microtube RCDs as in example (1). The seal in this instance was peelable and the samples were accessed by later removal of the film layer. Similar thermal

cycling experiments were conducted as in example (1), producing similar and satisfactory results.

In each example the thermally cycled RCDs resisted leakage throughout the complete experiment, indicated no loss of sample volume and revealed conclusively that the amplification reactions proceeded successfully.

Thus it can be seen that a closure embodying the invention provides a convenient liquid handling and prolonged storage means which can prevent all forms of sample contamination and spillage through the provision of a resilient and stable polymeric film barrier that can be punctured either by manual or robotic means to access the contained reagent mixture or simple liquid sample.

There may be provided a relatively simple sealing means and configuration for all current types of RCD that are used both in conjunction with existing PTC designs and elsewhere in a variety of purposes in the areas of biological sciences and clinical diagnostics, and which prevents sample loss at elevated temperatures during thermal cycling procedures. The need for an additional external oil overlay is dispensed with and a total barrier to both gas and moisture at all temperatures up to about 98°C is provided.

In brief summary the invention therefor comprises a range of hermetically sealable polymer films and method for their uses that can be used in conjunction with existing RCDs, or as provided here, through the provision of a substantially improved range of RCD's. All RCD's that utilize the present invention can be used in conjunction with simple heat or heat and pressure sealing devices to provide a means to resolve the aforesaid problems, i.e. use of oil overlays and prevention of sample loss, spillage and contamination in RCDs from either evaporation during elevated temperature thermal cycling or in general handling

and prolonged storage.



CLAIMS

1. A closure for a containment device, comprising a polymeric film, characterised by a heat resisting barrier layer 11 and a sealing layer 12, the barrier layer 11 having substantially low gas and moisture permeability.
2. A closure means according to Claim 1, characterised by said containment device 20 comprising a reagent containment device (RCD).
3. A closure means according to Claims 1 and 2, characterised by said layers 11, 12 being substantially adjacent one another in use.
4. A closure means according to Claim 3, characterised by the layers 11, 12 being combined by an adhesive 13.
5. A closure means according to any previous claim characterised by the barrier layer 11 being formed from a high temperature formable polymer.
6. A closure means according to any preceding claim, characterised by barrier layer 11 being selected from polyesters, polyamides, polyimides, and polymethylpentenes.
7. A closure means according to any preceding claim, characterised by the sealing layer 12 being selected from polypropylenes, polycarbonates, polystyrenes, polymethylmethacrylates, polyvinylchlorides, and amorphous polyesters.
8. A closure means according to any preceding claim, characterised by at least one additional layer 14 having heat and moisture insulating properties.

9. A closure means according to Claim 8, characterised by the additional layer(s) 14 comprising aluminium.
10. A closure means according to any preceding claim, characterised by being produced by lamination or co-extrusion.
11. A closure means according to any preceding claim, characterised by being in the form of a tape or strip 10.
12. A closure means according to any preceding claim, characterised by being in the form of a sheet.
13. A method for sealing containment devices, characterised by comprising applying a closure means 10 according to Claims 1 to 12.
14. A method according to Claim 13, characterised by applying heat.
15. A method according to Claim 13, characterised by further comprising applying pressure.
16. A containment device comprising containment means, and closure means according to any of Claims 1 to 12, characterised in that the containment means 20 and said closure means 10 are separate and chemically similar.
17. A containment device according to Claim 16, characterised by said containment means 20 having substantially a U-shape in section.
18. A containment device according to any of Claims 16 to 18, characterised by a plurality of containment means 10 and a carrier 10 comprising a plate or

tray, by the containment means 10 forming wells or depressions 44 in the plate or tray, and by an annular ring 42 substantially concentric with each well or depression 44.

20. A containment device according to any of Claims 16 to 18, characterised by comprising a microtube.

21. A containment device according to Claim 20, characterised by a tube having a sealing surface 21 comprising a holding flange, by substantially parallel side walls 23, and by a base section 24.

22. A containment device according to any of Claims 16 to 21, characterised by a capacity of between 5 micro litres and 100ml.

23. A containment device according to Claim 22, characterised by a capacity of about 250 micro litres.

24. A containment device according to Claims 14 to 21, characterised by a wall thickness of between 0.4 and 20.0mm.

25. A kit, comprising a containment device and closure means, characterised by said closure means is as according to any of Claims 1 to 12.

26. A kit according to Claim 25, characterised by dispensing means for said closure means.

27. A kit, comprising a containment device and closure means characterised by said closure means is as according to any of Claims 16 to 24.

28. A closure means according to Claim 27, characterised by dispensing means for said closure means.

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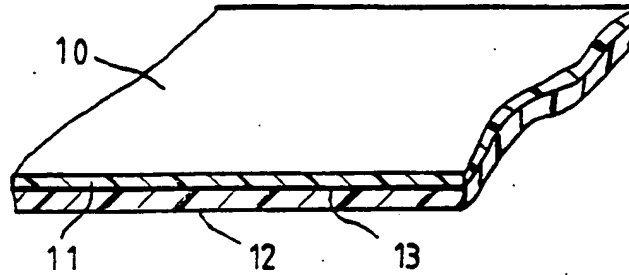


FIG. 1A

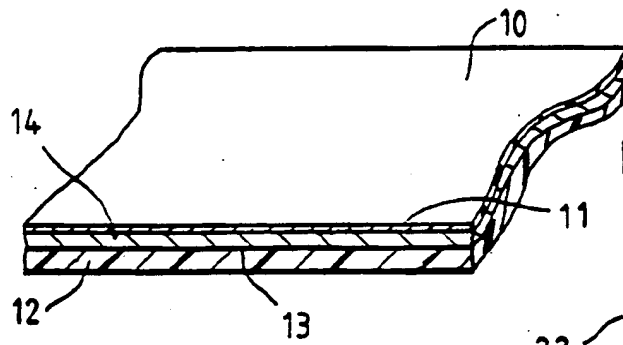


FIG. 1B

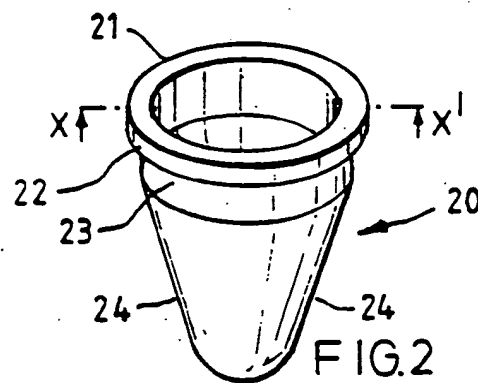


FIG. 2

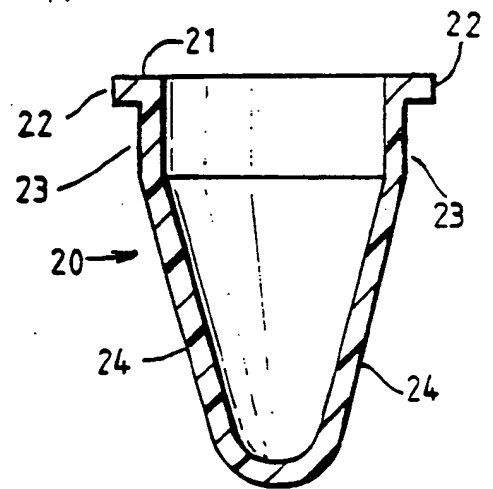


FIG. 3

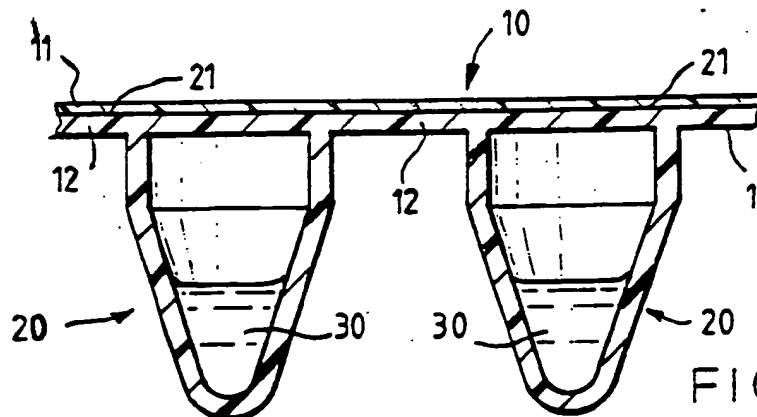
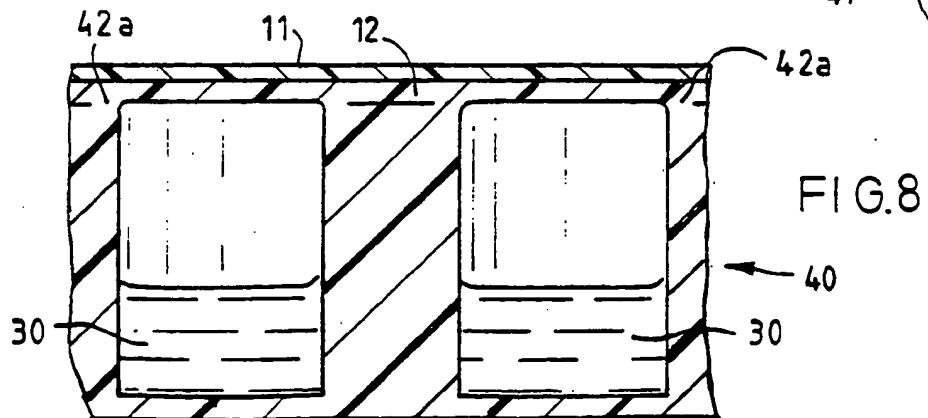
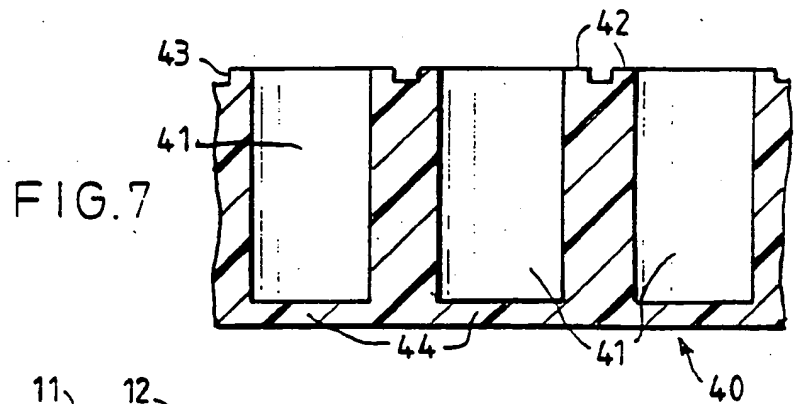
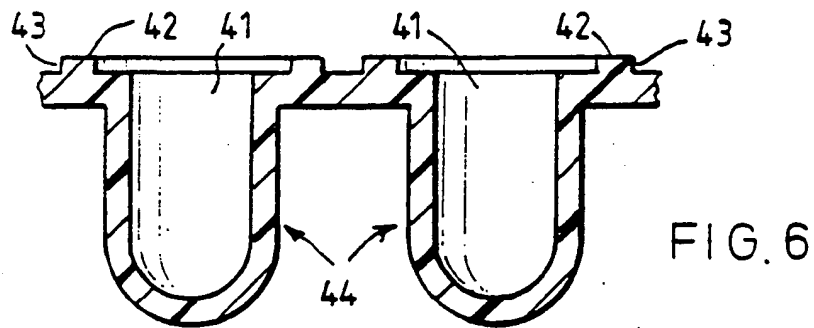
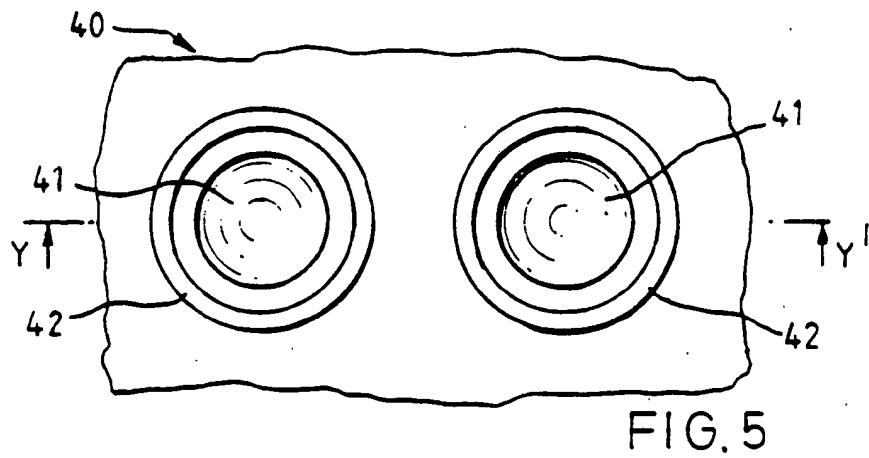


FIG. 4

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification<sup>5</sup>: B65D 77/20, B01L 3/00</p>	<p>A3</p>	<p>(11) International Publication Number: WO 94/12405 (43) International Publication Date: 9 June 1994 (09.06.94)</p>
<p>(21) International Application Number: PCT/GB93/02290 (22) International Filing Date: 8 November 1993 (08.11.93) (30) Priority Data: 9225307.9 3 December 1992 (03.12.92) GB (71) Applicant (for all designated States except US): TECHNE (CAMBRIDGE) LIMITED [GB/GB]; Duxford, Cambridge CB2 4PZ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): NORTON, David, Robert [GB/GB]; 6 Rotterdam Drive, Avon Village, Christchurch BH23 1HB (GB). REID, Mark, Anthony [GB/GB]; 7 Waterside, Mudeford, Christchurch BH23 3NE (GB). (74) Agent: JOHNSON, Terence, Leslie; Edward Evans &amp; Co., Chancery House, 53-64 Chancery Lane, London WC2A 1SD (GB).</p>		<p>(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 21 July 1994 (21.07.94)</p>
<p>(54) Title: CLOSURE MEANS, CONTAINERS AND METHODS OF CLOSURE</p> <p>(57) Abstract</p> <p>A closure means for containment devices such as reagent containment devices (RCDs) comprising a multilayer film (10) having at least two layers, an uppermost first layer (11) and adjacent to the first layer a second sealing layer (12), said first layer comprising a heat resistant barrier being substantially impermeable to gas and moisture. The film can be in the form of a strip or sheet and can be applied to for example microtubes, and multiwell plates or strips in order to hermetically seal them and prevent reagent loss through evaporation during treatment or spillage during handling or storage. There is further provided a method for sealing RCDs comprising applying a film as described using heat and/or pressure, forms of RCDs useful for said method, and kits comprising closure means and RCDs.</p> <div data-bbox="792 1171 1432 1453"> </div>		

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BG	Bulgaria	IE	Ireland	NZ	New Zealand
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CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
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CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				



## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/GB 93/02290A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 B65D77/20 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 B65D B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,3 946 871 (STURM) 30 March 1976	1,3
Y	see column 4, line 32 - line 58; figures 1-4	1-3
Y	see column 5, line 42 - column 6, line 24	5-7
Y	FR,A,2 110 030 (ASTEC INC.) 26 May 1972	1-3
A	see page 7, line 11 - line 34; figures 1-4	5,13
Y	EP,A,0 060 473 (ALUMINIUMWERKE AG.) 22 September 1982	5-7
	see page 2, line 33 - page 3, line 18; figure 1	
X	EP,A,0 292 386 (SOC. ALSACIENNE D'ALUMINIUM) 23 November 1988	1,3,4
	see column 3, line 37 - line 62; figures 1,2	
	see column 4, line 5 - line 23	
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

22 February 1994

Date of mailing of the international search report

16. 05. 94

Name and mailing address of the ISA

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Fax ( - 31-70) 340-3016

Authorized officer

VOLLERING, J

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 93/02290

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO,A,92 01513 (MAXPLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.) 6 February 1992 see page 11; figures 1-10 see page 22 see page 37</p> <p style="text-align: center;">---</p>	1,2,5,7, 13
A	<p>PATENT ABSTRACTS OF JAPAN vol. 8, no. 86 (P-269) (1523) 19 April 1984 &amp; JP,A,59 000 638 (TOKYO SHIBAURA DENKI K.K.) 5 January 1984 see abstract</p> <p style="text-align: center;">-----</p>	1

3

## INTERNATIONAL SEARCH REPORT

...ernational application No.

PCT/GB93/02290

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. Claim 2
  2. Claims 3-7, 10, 13-15 partially
  3. Claim 8,9
  4. Claim 11,13-15
  5. Claim 12-15
  6. Claim 16-24
  7. Claim 25-28
- See PCT/ISA/206 sent 17.3.94

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,2,3,4,5,6,7,10,13-15 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 93/02290

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		JP-T- 6500727	27-01-94